5 Using Marine Cryptides against Metabolic Syndrome

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5.1 MARINE CRYPTIDES

Proteolytic cleavage allows the release of peptides that are not the dead-end product of a digestion process, since they are able to exert some biological activity. They have been called "bioactive peptides," or more recently "cryptides," a neologism built from "crypted" and "peptides." Cryptic peptides were defined by Autelitano *et al.* (2006) as bioactive peptides hidden within the sequence of a parent protein. The release of these cryptic peptides has been shown to be prevalent in proteins associated with endocrine signaling and the extracellular matrix. As an example of an endocrine signaling protein, the prohormone proopiomelanocortin gives rise to diverse bioactive peptides: the adrenocorticotropic hormone, the β-lipotrophin, the γ-melanocyte-stimulating hormone, and others involved in a plethora of biological responses. Collagen α-chains, when proteolyzed by various matrix metalloproteinases (MMPs), serine proteases, and cathepsins, can release endostatin, arresten, or canstatin, all involved in angiogenesis regulation. Endogenous cryptides appear to play a role in modulating various biological processes, such as angiogenesis, immune function, and cell growth (Pimenta & Lebrun 2007). They are very diverse and are generated *in vivo* by a broad spectrum of proteases (MMPs, plasmin, kallikrein, angiotensin-converting enzyme (ACE), elastase, etc.). The proteolytic release of cryptides with novel activities represents an important mechanism for increasing the diversity of functions attributed to proteins.

This *in vivo* generation of cryptides by endogenous proteases has been mimicked *in vitro* and on a large scale to generate cryptides from terrestrial or marine proteins. It can potentially offer new opportunities for protein-based therapeutics. Cryptides may have related or even enhanced properties when compared to their corresponding native molecule or may possess completely unrelated biological effects, thus increasing the array of biological roles that can be associated with a given protein (or gene) (Pimenta & Lebrun 2007). The diversity of cryptides as functional molecules and their biological activities are actually only limited to those of pharmacological interest. Cryptides exhibit physiological effect and may contribute to human or animal health and wellbeing.

In this chapter, our attention will be focused on cryptides from marine protein sources. Several marine cryptides have already been isolated following hydrolysis of fish protein from muscle, skin, and frame. Shellfish and crustaceans contain crypteins: proteins able to release cryptides. They exhibit anticoagulant and antiplatelet properties *in vitro*, suggesting possible interactions with coagulation factors. Potent antioxidant activities have also been detected, in addition to immunostimulatory effects. Moreover, marine cryptides may take the form of hormone-like peptides and growth factors related to calcium absorption. Finally, marine cryptides exhibit antihypertensive activity, inhibiting the action

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of ACE—sometimes more so than other natural peptides from terrestrial sources (e.g. soy or milk). These cryptides have been shown to lower blood pressure in spontaneously hypertensive rats (SHRs) and humans (Kim & Wijesekara 2010; Harnedy & FitzGerald 2012).

This last biological activity is strongly related to the major human disease known as metabolic syndrome (MetS), which clusters hyperinsulinemia, insulin resistance, abnormalities in lipid metabolism, arterial hypertension, and obesity. Such metabolic disorders lead to increased risks of cardiovascular diseases (CVDs). A fish-enriched diet can efficiently reduce some risk factors, particularly through fish lipids and the presence of omega fatty acids (Aguilera *et al.* 2004; Nagao & Yanagita 2008). In addition to the well-known "good lipids," fish introduce interesting crypteins able to release, after their digestion, cryptides with all the biological activities just mentioned. The potential applications of fish protein hydrolysates (FPHs) are rising. Consequently, they have attracted particular interest in the reduction of MetS.

5.2 DEFINITION OF MetS

MetS clusters several metabolic abnormalities, including central obesity, dyslipidemia, hyperglycemia and hypertension. MetS has been found to be associated with atherosclerosis and the risk of coronary heart disease in the USA and Northern Europe (Bataille *et al.* 2006). Three different indexes define MetS: the National Cholesterol Education Program's (NCEP's), the World Health Organization's (WHO's), and the International Diabetes Federation's (IDF's); the latter is the most recent definition. Such indexes are presented in Table 5.1. There are major differences between the IDF's, WHO's, and NCEP's criteria for defining MetS: in the IDF's definition, abdominal obesity is a prerequisite, whereas in the NCEP and WHO definitions it is one among many factors, and its threshold is lower. In the WHO's definition, the first criterion is the level of fasting plasma glucose or fasting insulin associated with at least two abnormalities among obesity or central obesity, dyslipidemia, and arterial hypertension. In the NCEP's definition, MetS is considered present if at least three of the four requisites (dyslipidemia, arterial hypertension, diabetes, and obesity) are met.

The Prospective Epidemiological Study of Myocardial Infarction (PRIME), a multicenter prospective population-based cohort study, was designed to assess the contribution of various genetic and environmental exposures to the development of coronary heart disease (CHD) in two countries at contrasting risk of CHD (Ducimetiere *et al.* 2001). In this cohort study, IDF-defined, WHO-defined, or NCEP-defined MetS was found to be predictive of CHD risk in a population composed of male subjects from France and Northern Ireland (Bataille *et al.* 2006).

Table 5.1 Three different indexes defining metabolic syndrome: the National Cholesterol Education Program's (NCEP's), the World Health Organization's (WHO's), and the International Diabetes Federation's (IDF).

Metabolic abnormality	Measured criterion	IDF	NCEP	WHO
Dyslipidemia	Triglycerides	>1.70 mM	≥1.70 mM	≥1.70 mM
	HDL	<1.03 mM	<1.03 mM	<0.90 mM
Arterial hypertension	Blood pressure	≥130/85 mmHg	≥130/85 mmHg	≥140/90 mmHg
Diabetes	Fasting plasma glucose	≥5.6 mM	≥6.1 mM	≥6.1 mM/ or fasting insulin ≥13 IU/L[a]
Obesity	Waist circumference	≥94 cm[a]	>102 cm	BMI ≥ 30 kg/m^2 or waist-to-hip ratio (WHR) > 0.90

[a]Prerequisite.

Obesity is the most visible criterion and an early step in the etiological cascade leading to MetS. Obesity is defined as abnormal body weight and can be assessed by measuring waist circumference or by calculating the body mass index (BMI: kg body weight/height (m^2)). Individuals with a BMI $\geq$ 30 kg/m^2 are considered obese. This worldwide health problem is believed to be a result of modern life in rich, developed countries but is showing an increased prevalence in developing countries, with important social and economic implications (McLellan 2002). This situation requires urgent and efficient therapies. Data from the National Health and Nutrition Examination Survey (CDC 2005–06) indicate that the prevalence of obesity is 33–35% among US adults and 16% among children and adolescents aged 2–19 years (Ogden *et al.* 2007, 2008). In Europe, the prevalence of obesity ranges from 15 to 30% for men and from 11 to 34% for women (Maury & Brichard 2010). Obesity is not merely a cosmetic issue, being the etiologic factor in many serious diseases, including dyslipidemia, hypertension, stroke, myocardial infarction, type 2 diabetes, and some types of cancer (International Association for the Study of Obesity: www.iaso.org). Unbalanced diet and urbanization associated with genetic susceptibility have allowed the emergence of the obese phenotype. For this reason, the rapidly increasing level of obesity is likely to result in substantial health, financial, and social burdens across the world unless effective interventions are employed. Obesity has become a global problem with the rising popularity of junk food and Western lifestyles in many developing nations (particularly in urban locations) (International Obesity Task Force: www.iotf.org).

For many obese subjects, attempts to adhere to a healthy diet and lifestyle are not successful and bariatric surgery and/or pharmacotherapy are necessary. Between these two extremes there is room for an intermediate proposal, involving the consumption of functional foods that help to control energy intake or increase energy expenditure. Such foods could contain natural bioactive molecules with marine origins. Marine resources and in particular marine proteins show a promising pharmacotherapy potential: marine cryptides or cryptein hydrolysates could regulate the dyslipidemia involved *in fine* in CVDs. Hydrolysates from fish protein could also regulate blood glucose levels and hyperglycemia, which leads to type II diabetes. Moreover, they could reduce arterial hypertension and food intake, since they exhibit appetite suppressant activities. Figure 5.1 presents the activities of marine cryptides with regard to metabolic disorders linked to MetS.

5.3 POTENTIAL TARGETS FOR MARINE CRYPTIDES

5.3.1 Targeting dyslipidemia: modulating blood lipid profile

An unfavorable blood lipid profile is an important risk factor for various CVDs. There is a positive correlation between hypercholesterolemia and/or hypertriglyceridemia and the likelihood of developing CVDs. Not surprisingly, treatment for hyperlipidemia-accelerated diseases often includes the improvement of serum lipid distribution through diet modifications.

Several works have reported the importance of dietary proteins and protein hydrolysates in the regulation of cholesterol metabolism (Bakhit *et al.* 1994). Recent studies have reported that protein hydrolysates with marine origins may affect plasma cholesterol concentration and the metabolism of polyunsaturated fatty acids.

Wergedahl *et al.* (2004) compared the effect of FPH produced from salmon bone frame after filleting with that of soy protein and casein in obese Zucker rats. They showed that treatment with FPH affected both the fatty acid composition in liver and plasma and the triacylglycerol-rich lipoproteins levels. FPH lowered plasma cholesterol in Zucker rats by reducing the activity of acyl-CoA:cholesterol acyltransferase (ACAT), whereas soy protein did so by also affecting the secretion of fecal bile acids. FPH was found to have a potential role as a cardioprotective nutrient. This effect was additive to the effect already demonstrated for the fish oil. The combination of fish oil and FPH in the diet profoundly reduced the plasma cholesterol level, mainly due to its lowering high-density lipoprotein cholesterol (HDL-C) content (Wergedahl *et al.* 2009). Such findings were obtained in rats. Further studies are necessary to confirm that dietary FPH is also beneficial for plasma cholesterol levels in humans.

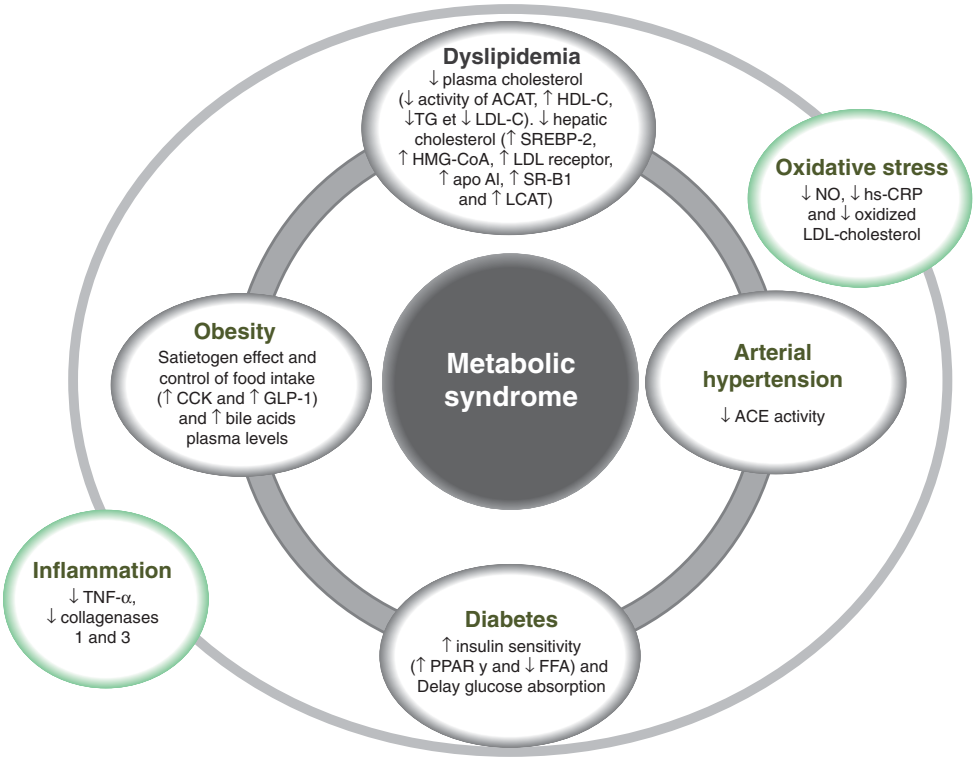
Dyslipidemia
↓ plasma cholesterol (↓ activity of ACAT, ↑ HDL-C, ↓TG et ↓ LDL-C). ↓ hepatic cholesterol (↑ SREBP-2, ↑ HMG-CoA, ↑ LDL receptor, ↑ apo AI, ↑ SR-B1 and ↑ LCAT)
Oxidative stress
↓ NO, ↓ hs-CRP and ↓ oxidized LDL-cholesterol
Obesity
Satietogen effect and control of food intake (↑ CCK and ↑ GLP-1) and ↑ bile acids plasma levels
Metabolic syndrome
Arterial hypertension
↓ ACE activity
Inflammation
↓ TNF-α, ↓ collagenases 1 and 3
Diabetes
↑ insulin sensitivity (↑ PPAR y and ↓ FFA) and Delay glucose absorption

Figure 5.1 Effects and mechanisms of action of marine peptides and hydrolysates in the pathogenesis of metabolic syndrome. ACAT: Acyl-CoA: cholesterol acyltransferase; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; SREBP-2: sterol regulatory element binding protein; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A reductase; apo AI: apolipoprotein A-I; SR-B1: scavenger receptor B1; LCAT: lecithin–cholesterol acyltransferase; NO: nitric oxide; hS-CRP: high-sensitivity C-reactive protein; CCK: cholecystokinin; GLP-1: glucagon-like peptide-1; ACE: angiotensin converting enzyme; TNF-α, tumor necrosis factor alpha; PPAR γ: peroxisome proliferator-activated receptor gamma; FFA: free fatty acid.

Recently, sardinelle (*Sardinella aurita*) protein hydrolysates (SPHs) obtained by treatment with crude enzyme preparations from *Bacillus pumilus* A1 (SPHA1) and *Bacillus mojavensis* A21 (SPHA21) and crude enzyme extracts from sardinelle viscera (SPHEE) have shown an *in vivo* hypolipidemic effect in rats. In this study, Wistar rats were fed either a standard laboratory diet, a cholesterol-enriched diet (1%), or a cholesterol- and SPH-enriched diet across 7 weeks. The hypercholesterolemic diet induced the elevation of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). Supplementation of a cholesterol-enriched diet with SPHs or whole sardinelle protein (WSP) at a concentration of 5% w/w increased the serum level of HDL-C and HDL-C/TC ratio and decreased the serum levels of TC, TG, LDL-C, and LDL-C/HDL-C ratio significantly compared to a cholesterol-enriched diet alone (Ben Khaled *et al.* 2012).

FPHs are not the only protein source capable of producing an effect on blood lipid levels. *Rhopilema esculentum*, a jellyfish commercially exploited in China for more than a thousand years, has been found to contain interesting cryptides with respect to MetS. Although its medicinal value has been long recognized, a very recent study has shown that oral administration of *Rhopilema esculentum* hydrolysate in SHR decreases TC and TG levels (Liu *et al.* 2012). Another experiment was performed with fish

protein from Alaskan pollock: *in vivo* experiments were conducted in rats fed diets containing either fish protein prepared from Alaskan pollock fillets or casein, which served as a control. Rats fed fish protein had higher concentrations of cholesteryl esters in the liver, a lower concentration of HDL-C, and lower plasma triacylglycerol concentrations than controls. They also had higher relative mRNA concentrations of sterol regulatory element-binding protein (SREBP)-2, 3-hydroxy-3-methylglutaryl coenzyme A reductase, LDL receptor, apo AI, scavenger receptor B1, and lecithin-cholesterolacyltransferase in their livers (Shukla *et al.* 2006). The data suggest that the fish protein from Alaskan pollock exerted distinct effects on plasma and liver lipids, which were at least partially caused by an altered expression of the hepatic genes involved in lipid homeostasis.

A limited number of marine-derived protein hydrolysates and peptides have been reported to have hypocholesterolemic and hypotriglyceridemic activities. However, published data offer a range of possible mechanisms of action underlying the ability of marine proteins to improve the blood lipid profiles of rats through reduction of TC, LDL-C, and HDL-C levels and an increase in fasting TG.

To date, most of the claimed physiological effects on lipid homeostasis of marine-derived bioactive protein hydrolysates and peptides have been observed *in vitro* or in rat model systems. Human clinical studies are limited or non-existent; most that do exist deal with fish lipid supplementation in the diet. Recent work has studied a possible link between fish consumption, low-level mercury (Hg), lipids, and inflammatory markers in children (Gump *et al.* 2012). Fish are the primary source of human exposure to Hg. This study documented an association between blood Hg, systemic inflammation, and endocrine disruption in humans, and concluded that a risk–benefit ratio for fish consumption by children should be determined. Environmental contamination of the sea should also be taken into account. Moreover, the optimal plasma levels of bioactive peptides leading to biological activity have yet to be determined. Therefore, human intervention studies are needed to verify the interesting results obtained through *in vitro* and/or animal experiments.

5.3.2 Targeting arterial hypertension

Hypertension, most commonly known as high blood pressure, is the leading cause of CVDs in the world, and one of the most common diseases affecting humans. Blood pressure is determined by cardiac output and vascular peripheral resistance, and is mainly regulated by the sympathetic nervous system (SNS), the rennin–angiotensin system (RAS), and the kidney and fluid balance mechanism.

Antihypertensive peptides may have multiple potential interaction sites, as blood pressure regulation is a complex system, involving many pathways.

Antihypertensive peptides are mainly discovered (and studied) through their inhibitory effect on ACE. A great number of ACE-inhibitory peptides have been isolated from the enzymatic digests of various fish and other marine organisms (Kim & Wijesekara 2010). The potency of these marine-derived peptides is expressed as the IC_{50} value, which is the ACE inhibitor concentration leading to 50% inhibition of ACE activity. When IC_{50} is measured, it can be used to compare the level of biological activity of hydrolysates or peptides across different studies. The *in vitro* potency of peptides isolated from marine protein hydrolysates is often compared to the potency of Captopril, the first synthetic ACE inhibitor to be designed (Cushman *et al.* 1977), or else to other peptides. For example, several peptides with IC_{50} values in the range 2.6–51.0 μM have been isolated from marine processing waste, mollusc, and crustacean protein hydrolysates (Byun & Kim 2001; Hai-Lun *et al.* 2006; Tsai *et al.* 2008). The *in vitro* potency of these peptides is comparable to the potency of the two blood-pressure-lowering milk-derived peptides, VPP and IPP, present in the commercially available antihypertensive functional beverages AmealS and Evolus (Harnedy & FitzGerald 2012).

Comparison between IC_{50}s can lead to false conclusions if all parameters are not considered, as the IC_{50} strongly depends on reaction type, volume, enzyme (quantity and quality), and, especially, the substrate used in the reaction. To date, synthetic substrates have been used to measure ACE activity and inhibition *in vitro*: Hippuryl-Histidyl-Leucine (HHL), N-[3-(2-Furyl)Acryloyl]-L-Phenylalanyl-Glycyl-Glycine (FAPGG), 2,4,6- trinitrobenzene sulphonate (TNBS), and o-aminobenzoylglycyl-p-nitrophenylalanylproline (Abz-Gly-Phe(NO2)-Pro; Abz) (Sentandreu & Toldra 2006). The conditions

of testing depend on the method used: high-performance liquid chromatography (HPLC), spectrophotometry, and spectrofluorometry are required when HHL, FAPGG, and Abz, respectively, are used. Natural substrates can also be used to examine ACE activity, such as angiotensin I (Ang I) and bradykinin (Ruiz-Giménez *et al.* 2011).

However, *in vitro* inhibition of ACE and the information obtained from simulated gastrointestinal systems are only an indicator of the potential for a peptide to act as a hypotensive agent *in vivo*. In order to exhibit physiological activity, a peptide must resist degradation and modification in the gastrointestinal and vascular systems and reach its target site in an active form. Only extensive *in vivo* studies can reliably assess the antihypertensive effect of a peptide (Harnedy & FitzGerald 2012).

In this regard, specific structural properties of ACE-inhibitory peptides play an important role. Most of these peptides are short, with a length ranging from two to nine amino acids. It has been demonstrated that di- and tripeptides, especially those with C-terminal proline or hydroxyproline residues, are generally resistant to degradation by digestive enzymes (Matsufuji *et al.* 1995; Vermeirssen *et al.* 2004). In addition, short peptides—generally ubiquitous—consisting of two or three amino acids, are absorbed more rapidly than free amino acids (Erdmann *et al.* 2008). An interesting example comes from LKPNM, a peptide isolated from the thermolysin digest of "Katsuo-bushi," a Japanese traditional food processed from dried bonito, which possesses potent inhibitory activity against ACE (Fujita & Yoshikawa 1999). Following hydrolysis of LKPNM to LKP by ACE, an eightfold increase in ACE-inhibitory activity is observed. LKPNM shows almost the same antihypertensive activity as Captopril on a molar basis after a single oral administration in SHR, and exerts long-lasting antihypertensive activity comparable to that of Captopril. In a small-scale clinical study, the thermolysin digest of dried bonito evidenced long-lasting antihypertensive activity following oral administration in hypertensive and borderline-hypertensive subjects. Incidentally, this digest, called "Katsuo-bushi oligopeptide," was officially Foods for Specified Health Use (FOSHU)-approved as by the Ministry of Health and Welfare in Japan (Fujita *et al.* 2000). Numerous *in vivo* studies in SHR, as well as in hypertensive human volunteers, have demonstrated that several ACE-inhibitory peptides derived from marine sources significantly reduce blood pressure after both intravenous and oral administration (Kim & Wijesekara 2010).

ACE-inhibitory peptides can be applied as either an initial or a supplemental treatment in mildly hypertensive individuals. Due to the incomplete protection they provide, however, Ang II receptor blockers (ARBs) have also been developed. The combination of renin inhibitors and ACE inhibitors/ARBs has recently emerged as a strategy for the therapeutic treatment of hypertension. Synthetic peptides and peptidomimetic compounds previously developed to effectively inhibit renin activity *in vitro* have been shown to reduce blood pressure in animals and humans (Harnedy & FitzGerald 2012). However, these compounds can only be intravenously (IV) administered. It appears there is currently no information regarding the inhibition of renin activity by marine processing waste-, mollusc-, and crustacean-derived protein hydrolysates or peptides. Flaxseed protein hydrolysates and the dipeptides IR, KF, and EF isolated from pea protein hydrolysates have been shown to inhibit renin and ACE *in vitro* (Li & Aluko 2010). Therefore, mining of marine sources for peptides that inhibit both renin and ACE could potentially provide opportunities to develop a natural antihypertensive agent with a dual action.

Peptides derived from marine sources may influence blood pressure through mechanisms other than the established ACE inhibition. Indeed, it has been reported that the release of vasodilatory substances such as prostaglandin I_2, nitric oxide (NO), and carbonic oxide (CO) can also contribute to the blood-pressure-lowering effects of various ACE-inhibitory peptides (Erdmann *et al.* 2008). Furthermore, inhibition of an enzyme involved in the cascade generation of Ang II from marine cryptides (such as chymase or Cathepsins A, D, or G) may provide an additional antihypertensive effect. Identification of potential peptides derived from marine proteins that are able to exert an inhibitory effect on the activity of these enzymes may provide a new approach to the reduction of blood pressure and other Ang II-related metabolic disorders.

Marine-derived bioactive peptides have potential for use as functional ingredients in nutraceuticals and pharmaceuticals, due to their effectiveness in both the prevention and the treatment of hypertension.

Moreover, cost-effective and safe natural health products can be produced from marine bioactive peptides. A large number of *in vitro* experiments have provided evidence that fish-based peptides have a greater antihypertensive effect than many other proteins sources. However, further *in vivo* evidence is still needed before they can be applied to human health promotion.

5.3.3 Targeting diabetes

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Hyperglycemia, or raised blood glucose, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems. Heart disease, MetS, and type II diabetes have in common the increased concentration of circulatory cytokines as a result of inflammation. Inflammatory cytokines are produced by various cell types and secreted into the circulation, where they regulate various tissues through their local, central, and peripheral actions. The best strategy for favorably altering the inflammatory response is still a matter of debate (Calle & Fernandez 2012).

In type I diabetes, the immune system attacks the cells that produce insulin, while in type II diabetes it makes cells more resistant to insulin. This induced insulin resistance is linked to chronic inflammation and to the release of the pro-inflammatory cytokine tumor nuclear factor alpha (TNF-α) (Shoelson 2006).

It has been observed that in inflammatory diseases, the antioxidant defense system is compromised, as evidenced by increased markers of oxidative stress and decreased levels of protective antioxidant enzymes. Therefore, dietary antioxidants, and particularly those of marine origin, may be protective against the development of inflammatory diseases (D'Orazio *et al.* 2012). The exact mechanism by which peptides display antioxidant activity is not fully understood. However, it has been shown that protein hydrolysates and peptides can act as radical scavengers and as well as transition metal chelators, and exert antioxidant activities against enzymatic and non-enzymatic peroxidation of lipids and fats (Sarmadi & Ismail 2010). Furthermore, peptides have been shown to induce specific genes encoding for endogenous non-enzymatic antioxidant components and enzymatic systems in cultured cell models (Erdmann *et al.* 2006).

Marine waste, mollusc, and crustacean protein hydrolysates contain antioxidant peptides that may have a role in the prevention of oxidation-related damages (Ryan *et al.* 2011; Najafian & Babji 2012). Other peptides have antidiabetic properties (discovered rather recently), exerted through modulation of insulin sensitivity and glucose uptake. Lavigne *et al.* (2001) tested the hypothesis that fish protein may represent a key constituent of fish (including cod, shark, and salmon) with glucoregulatory activity. The observed beneficial effects of cod protein occurred without any reductions in body weight gain, adipose tissue accretion, or expression of TNF-α in fat and muscle. Feeding cod protein to high-fat-fed rats prevented obesity-induced muscle insulin resistance, at least in part through a direct action of amino acids on insulin-stimulated glucose uptake in skeletal muscle cells. Further studies exploring the cellular mechanisms of this increase of insulin sensibilization concluded that dietary cod protein restored insulin-induced activation of phosphatidylinositol 3-kinase/Akt and GLUT4 translocation to the T-tubules in the skeletal muscle of high-fat-fed obese rats (Tremblay *et al.* 2003).

The beneficial effects of salmon skins were assessed using a rat model of type II diabetes. Zhu *et al.* (2010c) studied β-cell dysfunctions, which are one of the important characteristics of this type of diabetes. Oligopeptides from marine salmon skin were able to significantly reduce the levels of fasting blood glucose and the frequency of apoptosis of β-cells in the pancreatic islet. Moreover, the effect was thought to be mediated by down-regulation of type II diabetes-related oxidative stress and inflammation. A treatment based on marine peptides obtained from wild fish meat hydrolyzed by pepsin, chymotrypsin, trypsin, and pancreatic lipase was given to Chinese patients with type II diabetes (Zhu *et al.* 2010a). Interesting results on markers of metabolic disorders were obtained. For example, significantly reduced levels of fasting blood glucose, human glycated hemoglobin A1c, and fasting blood insulin were observed. Marine fish hydrolysate treatment improved glucose and lipid metabolism in diabetic (Zhu *et al.* 2010a) and in diabetic and hypertensive patients (Zhu *et al.* 2010b).

Huang & Wu (2010) purified and characterized an antidiabetic peptide from shark (*Chiloscyllium plagiosum*). The purified peptide S-8300 (8200 Da) was isolated following homogenization of fresh shark livers. The mixture was heated at 95 °C and centrifuged. After various steps of ultrafiltration, dialysis, and chromatography, just one active fraction was isolated. Biological activity was assessed using mouse insulinoma β-cell line (NIT-1), on which streptozotocin had the adverse effect of insulin secretion. The S-8300 peptide was found to protect the structural integrity of cells and markedly decreased the level of fasting plasma glucose in rats. The peptide was stable toward high temperature (95 °C, 30 minutes) and between pH 3 and 9, but was totally inactivated by proteinase K and by trypsin digestion, preventing its direct use as a functional ingredient.

Some potential functional ingredients capable of improving blood glucose levels have been studied. A sardine muscle enzymatic hydrolysate was shown to ameliorate glucose tolerance in stroke-prone SHR; the observed effect was linked to the presence of ACE-inhibitory peptides (Otani *et al.* 2009). This last finding indicates that marine cryptides might be active toward at least two pathologies of the MetS. Further studies on the synergistic actions and multifunctional effects of marine cryptides will certainly confirm their potential against the linked MetS pathologies.

5.3.4 Targeting obesity

Obesity is determined by an increase in body weight and more specifically in adipose tissue and fat. It is a multifactorial syndrome that results from a combination of genetic, environmental, psychological, social, and cultural factors. Genes involved in appetite and body weight regulation have been identified, but their contribution to human obesity seems very modest (less than 5%) (Barceló-Batllori & Gomis 2009). Obesity implies dysfunctions in metabolic rate, lipid metabolism, adipocyte function, and thermogenesis or energy intake (Barceló-Batllori & Gomis 2009). Various techniques, including proteomics, have been used to identify targets for potential therapies. Comprehension of the signal integration between adipose tissue, the peripheral organs, and the CNS that leads to energy homeostasis regulation has allowed the synthesis of numerous molecules with potential utility against obesity (Barceló-Batllori & Gomis 2009).

At present, there are no centrally-acting drugs on the market for the treatment of obesity in Europe. Only Orlistat (Roche), a lipase inhibitor, is actually marketed. In the USA, the only CNS drugs that can be used to treat obesity are sympathomimetics and appetite suppressants, which are only approved as short-term ($\leq$12 weeks) treatments. Some years ago, D-amphetamine and methamphetamine were prescribed as appetite suppressants, but their frequent misuse and abuse led to their becoming highly restricted controlled drugs. Other drugs were withdrawn from the market when their prolonged use was linked to life-threatening primary pulmonary hypertension and cardiac valvulopathy (Heal *et al.* 2012). The much publicized adverse effects of such drugs opened the door to "natural" molecules—whose effects are less rapid—and to traditional methods of weight loss.

The major contributors to the increased level and severity of obesity are easy access to a diet rich in fat, sugar, and salt and a sedentary lifestyle. Thus, the first means of reducing obesity is to decrease food intake and increase calorie expenses. Once absorbed, food is converted to nutrients and stored under TG form in adipose tissue until it is used. The long-term regulation of maintenance of body weight is conducted by several endocrinal signals, such as insulin and leptin. This regulation is coupled with short-term signaling related to meal size, such as cholecystokinin (CCK). The release of CCK in the lumen leads to intestinal motility, gall bladder contraction, inhibition of gastric emptying, and stimulation of pancreatic secretion (Strader & Woods 2005). Thus, CCK contributes to maintaining appropriate size meal and regulating daily energy intake.

During digestion, bile acids are secreted from cholesterol by the liver. These are the main component of bile. It is likely that the hepatic supply of taurine and glycine affects bile acid conjugation and secretion, which can interfere with hepatocyte receptors. Targeted disruption of the gene encoding for this receptor leads to increased fat accumulation and body weight (Maruyama *et al.* 2006). Thus, the perturbation of bile acid secretion is also an attractive drug target for treatment of human metabolic disease.

5.3.4.1 *Targeting obesity by increasing satiety*

Satiety signals are reported to include amino acid precursors or neurotransmitters, serotonin, and catecholamine (noradrenaline and dopamine). The levels of circulating metabolites and hormones (amino acids, glucose, and insulin) and gut factors (distention, osmolarity, and gastrointestinal hormones such as CCK) also modulate satiety. Some studies have been carried out in order to demonstrate that hydrolysates from marine species are able to have an effect on satiety.

In 1991, Uhe *et al.* (1991) compared postprandial satiety and plasma amino acid, insulin, and glucose concentrations in six lean male subjects following ingestion of three types of protein (lean topside steak, chicken breast with skin removed, and fish (*Mustelus antarcticus*)). Satiety was greater after the fish meal. According to these authors, this greater satiety might be correlated with serotoninergic activity or might be attributable to differences in the postprandial tryptophan–large neutral amino acid (LNAA) ratio and/or differences in digestibility reflected in the longer time required for the plasma amino acids to peak following the fish meal. Other putative signals of satiety (insulin, glucose, and the tryptophan–LNAA ratio) were not different following consumption of the three meals used in this study. Differences in concentrations of taurine and methionine (which were greater after the fish meal) were also found, without known biological significance.

Another study focused on the effect of protein hydrolysate from smooth hound (*Mustelus mustelus*), a houndshark of the family Triakidae, in the regulation of components of food intake control, such as satiety. In this research, protein hydrolysate was produced by intestinal digestive proteases from the same species. The amino acid analysis by gas chromatography/mass spectrometry (GC-MS) showed that the hydrolysate was rich in leucine, alanine, glycine, threonine, serine, lysine, and glutamic acid. The molecular weight of the peptides were estimated to be between 200 and 2500 Da. The biological *in vivo* capacities of smooth hound hydrolysate in rats were evaluated, and it was found that the food intake and the body weight of the rats significantly decreased at the end of treatment (Bougatef *et al.* 2010). The satietogen effect observed was not significantly correlated with insulin and CCK concentration in the plasma of rats nourished with smooth hound hydrolysate and was probably mediated by other hormones. However, the reduction of food intake mediated by anorectic neuropeptides is one possible mechanism for the beneficial effects of fish hydrolysate in appetite regulation.

Appetite regulation is highly complex and involves a large number of orexigenic and anorexigenic peptide hormones. These are small, processed, secreted peptides derived from larger prepropeptide precursors. These peptides are important targets for the development of therapies for obesity (Seim *et al.* 2012). The long-term regulation of body weight is integrated by several endocrinal signals, such as insulin and leptin. This regulation is coupled with short-term signals related to meal size, such as CCK, which contributes to maintaining appropriate meal size and to regulating daily energy intake. Human CCK-secreting cells have been shown to exhibit similarities to the enteroendocrine STC-1 cell line, allowing the development of *in vitro* tests for research into peptides that are able to increase CCK secretion and are therefore linked to appetite suppression. FPHs from blue whiting (*Micromesistius poutassou*) muscle and brown shrimp (*Penaeus aztecus*) heads have been shown to greatly stimulate CCK-releasing activity from STC-1 cells (Cudennec *et al.* 2008). For example, when a 1% wt/vol of brown shrimp hydrolysate was incubated for 2 hours, STC-1 cells were able to release 160.7 pM of CCK, while albumin egg hydrolysate used as positive control allowed the release of 67.6 pM of CCK. The blue whiting hydrolysate also allowed the secretion of a high amount of CCK. Partial purification confirmed the peptide composition of the biologically active fractions. Molecular masses were estimated at less than 1500 Da. The authors found CCK-stimulating peptides in the entire molecular weight fraction obtained after gel permeation, and they explained this fact by the existence of different-molecular-weight peptide generated from the same parent protein. *In vitro* results on STC-1 cell lines were confirmed by *in vivo* experiments on rats. Indeed, Cudennec *et al.* (2012) demonstrated that hydrolysates produced from blue whiting muscle possess satiating properties. Such commercial hydrolysate was not only able to enhance CCK and glucagon-like peptide 1 (GLP-1) secretion in the STC-1 cell line but also to reduce the short-term food intake of rats in correlation with an increase in the CCK and GLP-1 plasma levels. Moreover, it was demonstrated that the chronic administration of

the hydrolysate led to a decrease in body weight gain. To date, no peptide sequences allowing CCK secretion and exhibiting satietogenic effect have been characterized, but the marine cryptides involved may be quite small.

5.3.4.2 *Targeting obesity-modulating bile acid secretion and hormonal control of gallbladder filling*

Bile is composed primarily of bile acids, cholesterol, and phospholipids. It is produced by the liver and stored in the gallbladder. In response to the ingestion of food, the hormone CCK is secreted from enteroendocrine cells in the duodenum in order to promote the contraction of the gallbladder and the release of bile into the small intestine, where it facilitates the absorption of lipophilic nutrients (Choi *et al.* 2006).

Taurine and glycine supplementation can affect bile acid metabolism. Bile acids are conjugated with glycine and taurine following their synthesis, which increases their solubility and enhances biliary secretion. Different sources of dietary protein have been investigated in order to explore to what extent they affect bile acid metabolism in young rats. Liaset *et al.* (2009) analyzed saithe FPH rich in taurine and glycine to check its ability to modulate the systemic effects of bile acids. Rats fed a diet containing this hydrolysate showed a markedly elevated fasting bile acid plasma levels compared to those fed soy or casein. The authors also showed that the rats had reduced liver lipids and fasting plasma TG levels. Visceral adipose tissue mass was reduced. Thus, the addition of FPH in foods might lead to a decrease of adipose tissue in rats. To date, no studies have been undertaken on human beings to demonstrate that fish muscle hydrolysate consumption can help decrease visceral adipose tissue.

Understanding the factors that control appetite increases the number of potential targets for marine cryptides. Concerning bile acids and their role, it has been found that they can activate a G-protein-coupled receptor designated as TGR5, which is expressed in brown adipose tissue and muscle (Kawamata *et al.* 2003). TGR5 triggers an increase in energy expenditure and may attenuate diet-induced obesity (Watanabe *et al.* 2006). TGR5 is also expressed in enteroendocrine cells in the intestine, where its pharmacologic activation induces energy expenditure and improves insulin sensitivity in rodent models of obesity (Thomas *et al.* 2009). More recently, it has been shown that TGR5 is highly expressed in gallbladder, where it stimulates the latter's filling with bile (Li *et al.* 2011). TGR5 is an attractive drug target for treatment of obesity and type II diabetes, but no studies involving marine cryptides acting against this receptor have yet been conducted.

In terms of regulation of food intake, ghrelin represents one of the key players. Ghrelin intertwines two drives for feeding: metabolic signals and pleasure signals. This 28-amino-acid peptide is the only circulating orexigenic peptide. It acts not only as a hunger signal but also as an adiposity signal. Ghrelin stimulates adipogenesis and promotes storage of energy as fat. It also regulates the frequency of meals (Depoortere 2009). In addition, ghrelin has direct effects on components of the reward system and increases the appetite value of food. Compounds that interfere with ghrelin signaling may therefore become powerful tools in the treatment of such conditions as obesity. The development of selective high-affinity ghrelin-receptor antagonists is thus of interest. Compounds that are under development for the treatment of weight disorders through the neutralization of ghrelin or through acting as a ghrelin antagonist are under preclinical status (Depoortere 2009). Treatment with ghrelin antagonists may produce side effects affecting mood and memory (Depoortere 2009). No marine cryptides have been tested as ghrelin antagonists, but they might prove to be an alternative with no side effects and good tolerance.

5.3.5 Others possible marine cryptide targets related to MetS

As has been already mentioned, MetS defines the association of four metabolic disorders: dyslipidemia, arterial hypertension, diabetes, and obesity. These four pathologies can be "treated" in various ways by the use of isolated marine cryptides or hydrolysates, as described in this chapter.

In addition to these pathologies, a number of complex social and environmental factors affect ill subjects. Stress is one such factor, and stress-induced eating has received substantial attention in the obesity-related literature (Moore & Cunningham 2012). The reduction of stress by the absorption of protein hydrolysate has been demonstrated for a milk protein hydrolysate named Lactium®, commercialized by Ingredia (Arras, France; www.ingredia.com) (Miclo *et al.* 2001; Bénézech *et al.* 2009). Such products have been prepared from FPHs and commercialized (Guerard *et al.* 2010).

Other potential marine cryptide targets of interest include the adipocyte life cycle, which is involved in adipose tissue size and mass expansion; calcium consumption and absorption, an increase of which some studies have shown could help to decrease obesity; or protein hydrolysates generated from fish and shellfish proteins, which may have relevance to CVD risk reduction, since some have anticoagulant and antiplatelet activity.

5.3.5.1 *Targeting stress*

From a physiological standpoint, stress is defined by the activation of the hypothalamic–pituitary–adrenal (HPA) axis (Gillespie & Nemeroff 2007). Stress leads to the release of epinephrine and glucocorticoids from the adrenal gland. Once released, these can produce a wide variety of effects in the periphery, ranging from increases in cardiovascular activities to decreases in gastrointestinal and immune function to increases in energy mobilization. Stress hormones also play critical functional roles in the CNS. Responses to acute stressful events are protective and adaptive in nature. Chronic stress elicits neurochemical, neuroanatomical, and cellular changes that may have deleterious consequences for higher brain functioning (McEwen & Seeman 1999). For example, while exposure to acute stress facilitates memory formation and consolidation, chronic stress or chronic exposure to stress levels of glucocorticoids impairs cognitive performance (McEwen 2007).

The stress–eating–obesity relationship has not been fully elucidated in animal or human populations, but psychological stress is a hypothesized contributor to increased emotional eating and obesity (Moore & Cunningham 2012). If acute stress (short duration) typically activates hormonal events that decrease the quantity of food intake, chronic stress (long duration) is of great interest in the etiology of obesity and other metabolic illnesses (Moore & Cunningham 2012). The reduction of stress in animals is difficult to prove. The first step often consists of a demonstration of the existence of antioxidant molecules. Antioxidant property is defined by the capacity to reduce oxidative stress. The term "antioxidant" is defined as "any substance that, when present at low concentration compared to that of oxidizable substrate, significantly delays or inhibits oxidation of that substrate." Antioxidants can act at different levels in an oxidative sequence by retarding the formation of free radicals (preventive antioxidants) or by introducing substances that compete for the existing radicals and remove them from the reaction medium (chain-breaking antioxidants). Antioxidant peptides of marine origin are well documented and exist in many commercial products with nutraceutical applications, such as Stabilium 200, an Atlantic fish autolysate from Compagnie Générale de Diététique-Yalacta (Caen, France; www.yalacta.com), and PROTIZEN®, a white fish hydrolysate from Copalis (Le Portel, France; www.copalis.fr), both of which have a relaxing effect. Fortidium LIQUAMEN® is a fish autolysate of white fish (*Molva molva*) from Duchange laboratories (Mondeville, France; www.biothalassol.com) that exhibits multiple effects, including a reduction of oxidative stress, a lowering of the glycemic index, and a reduction of stress (Guerard *et al.* 2010).

Such antistress properties concern cellular stress and not human stress as previously defined. However, it has been reported that low-molecular-weight peptides consisting of three to seven L-amino acids sometimes exhibit anxiolytic-like activities following oral administration in behavioral pharmacological tests in mice (Hirata *et al.* 2007). A short sequence consisting of tyrosyl leucine (YL) had potent anxiolytic-like activity following oral administration at low dose (Kanegawa *et al.* 2010). In order to ascertain its mechanism of action, Kanegawa *et al.* (2010) investigated whether the anxiolytic-like activity of YL was mediated by activation of serotonin 5-HT1A receptor, dopamine D1 receptor, and c-amino butyric acid type A (GABAA) receptor, which are known to play an important role in anxiolytic-like activity. These authors found that YL exhibits a potent dose-dependent anxiolytic-like

activity (0.1–1.0 mg/kg, intraperitoneal (IP)) comparable to that of diazepam in the elevated plus-maze test that is classically performed for the assessment of anxiety-related behavior in mice. YL was also active following its oral administration (0.3–3.0 mg/kg). However, the peptide LY and the mixture of amino acids Tyr and Leu were inactive. The anxiolytic-like activity of YL was inhibited by antagonists for serotonin 5-HT1A, dopamine D1, and GABAA receptors. The order of their activation was 5-HT1A, D1, GABAA receptors, as demonstrated by selective agonists and antagonists.

To date, no studies concerning the anxiolytic properties of FPH have been conducted. The presence of anxiolytic peptides like YL should be assessed in commercial products with relaxing or antistress effects.

5.3.5.2 *Targeting adipocyte life cycle*

The regression of adipose tissue may be key to the reduction of obesity. The important cosmetic issue of obesity has led to the development of numerous products for topic application or dietary supplemention. Such products contain mainly caffeine or polyphenols. For example, Bliss Fatgirlslim® cream contains encapsulated caffeine (www.sephora.com) and Vichy Laboratories' (Saint-Ouen, France; www.vichy.fr) Celludestock® cream is 5% caffeine. A citrus-based polyphenolic dietary supplement called Sinetrol® has a strong lipolytic effect mediated by cAMP-phosphodiesterase inhibition (Dallas *et al.* 2008).

Excessive caloric intake without a corresponding rise in energy expenditure promotes adipocyte hyperplasia and adiposity. The rise in adipocyte number is triggered by signaling factors that induce conversion of mesenchymal stem cells (MSCs) to preadipocytes, which differentiate into adipocytes. MSCs, which are recruited from the vascular stroma of adipose tissue, provide an unlimited supply of adipocyte precursors (Tang & Lane 2012). Selectively decreasing such adipocyte precursors, interfering with the conversion of MSCs to preadipocytes, and interfering in the conversion of preadipocytes to adipocytes may also allow a decrease of the adipose mass.

Rodbell's (1964) report involving the attainment of intact fat cells through collagenase digestion of adipose tissue revolutionized studies of the hormonal regulation and metabolism of the fat cell. Fat cells are now used as a tool for discovering and investigating various cellular mechanisms. Two kinds of fat cell can be used: mature fat cells from bariatric surgery, which lead to *ex vivo* experiments, and fat cells originating from adipose cell lines (from rodents or humans). Dormant preadipocytes exist in the stroma-vascular fraction of the adipose tissue of rodents and humans; cell culture systems have proven to be valuable models for the study of the processes involved in the formation of new fat cells (Lafontan 2012).

The adipose tissue can also be a target for marine cryptides. Studies have shown that ACE-inhibitory peptides from whey proteins are able to decrease the cultured preadipocyte number. A slight lipolytic effect has also been observed (Hammé *et al.* 2010). Since this biological activity is commonly described for marine cryptides, it is likely that ACE-inhibitory peptides from marine sources will exhibit some interesting potential in decreasing fat tissue. The effect on adipocyte hypertrophy could be evaluated through measurement of the residual lipid content of adipocytes or through lipolysis after short or long exposure. Apoptotic and/or antiproliferative cryptides should also be investigated in order to control adipocyte hyperplasia.

Discovering such cryptides is an exciting challenge that could lead to an interesting alternative to the caffeine and polyphenols currently involved in the formulation of cosmetics aimed at decreasing body weight and body fat.

5.3.5.3 *Targeting calcium*

The major source of calcium for humans is the diet, and the most common and trusted source of calcium is milk or dairy products. Casein phosphopeptides (CPPs) derived from the intestinal digestion of casein have been shown to enhance bone calcification in rats (Lee *et al.* 1980). Such CPPs have the capacity to chelate calcium and prevent the precipitation of calcium phosphate salts, thereby increasing

the amount of soluble calcium available. However, in Asian countries milk consumption is very limited due to lactose indigestion and intolerance. Protein hydrolysate and cryptides from marine processing waste, mollusc, and crustacean proteins also exhibit calcium-binding activity. Several calcium-binding peptides have been isolated and characterized from the skeletons discarded from industrial processing of hoki (*Johnius belengerii*). These skeletons were digested by heterogeneous enzymes extracted from the intestine of a carnivorous fish (also discarded from industrial processing), bluefin tuna (*Thunnus thynnus*). The effective biodegradation of the hoki bone matrices, comprising collagen, noncollageneous proteins, carbohydrates, and minerals, was observed. A fish bone phosphopeptide (FBP) containing phosphorus (23.6%) was isolated from the hoki bone hydrolysates. The FBP, with a molecular mass of 3.5 kDa, was able to maintain 41.1 mg/l soluble calcium in 20 mM phosphate buffer (pH 7.8) without the formation of insoluble calcium phosphate. The enzymatic degradation of the bone could be utilized as a nutraceutical with a potential calcium-binding activity, leading to the high bioavailability of calcium (Jung *et al.* 2005).

In another study, a low-molecular-weight peptide with a high affinity to calcium was recovered from pepsinolytic hydrolysates of *Theragra chalcogramma* backbone discarded from fish processing. *T. chalcogramma* backbone peptide (TBP), Val-Leu-Ser-Gly-Gly-Thr-Thr-Met-Ala-Met-Tyr-Thr-Leu-Val (1442 Da), exhibited the highest affinity to calcium ion on the surface of hydroxyapaptite (HA) crystals. *In vitro* calcium-binding assay elucidated that TBP can solubilize a similar content of calcium-like CPP. The utilization of fish backbones as a nutraceutical material with a high solubility for calcium should be developed in order to reduce the risk of osteoporosis in people with lactose intolerance (and particularly in Asians) (Jung *et al.* 2006). In addition to the reduction of risk of osteoporosis, several epidemiological studies suggest that calcium affects weight loss. However, calcium is not a magic bullet in the battle against obesity, although it may play a small but significant role, as suggested by Schrager (2005) and Zemel (2002, 2005). Considering that FPH can also bind calcium and could exert some role in other obesity parameters, it is expected that some studies will demonstrate in the near future that fish protein can be included in weight-loss dietary programs.

5.3.5.4 *Marine cryptides with anticoagulant or antiplatelet aggregation properties*

Hydrolysates generated from fish and shellfish proteins with potential relevance to CVD risk reduction include hydrolysates with anticoagulant and antiplatelet activity. Anticoagulant marine bioactive peptides have been isolated from marine organisms such as marine echiuroid worm (Jo *et al.* 2008), starfish (Koyama *et al.* 1998), blue mussel (Jung & Kim 2009), blood ark shell (Jung *et al.* 2001), and yellow fin sole (Rajapakse *et al.* 2005). The anticoagulant activity of those peptides was determined by the measurement of activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) assays. The activity observed was compared with the time obtained for heparin, a commercial anticoagulant.

A 12 kDa single-chained monomeric anticoagulant peptide has been purified from yellow sole frame protein hydrolysates (Rajapakse *et al.* 2005). This peptide was bound, and formed an inactive complex with factor XIIa. Furthermore, it exhibited antiplatelet activity by inhibiting the platelet membrane integrins GPIIb/IIIa. No research has been carried out to assess the efficacy of this peptide *in vivo*. Jung *et al.* (2007) characterized a 7.7 kDa anticoagulant polypeptide termed "TGAP" with a partial sequence from the muscle protein of the South Korean bivalve *Tegillarca granosa*. The anticoagulant polypeptide, which demonstrated low *in vitro* cytotoxicity to venous endothelial cells, specifically inhibited the blood coagulation factor Va, as well as the molecular interaction between factor IIa and factor Va in a concentration-dependent manner ($IC_{50} = 77.9$ nM), resulting in prolonged PT. Therefore, marine proteins are worthwhile target substrates for further anticoagulant peptide mining. However, the demonstration of beneficial health effects associated with anticoagulant activities needs to be confirmed by human studies before the health claims of marine proteins and peptides can be recognized.

5.4 CONCLUSION

Marine organisms such as fish and shellfish and marine-derived wastes and byproducts are rich sources of structurally diverse bioactive nitrogenous components called cryptides. Their sequences are hidden in the sequences of parent proteins and they can exert a wide range of biological activities. Many studies have been conducted into the physiological effect of these cryptides in humans. Considering the pathologies clustered in MetS—insulin resistance, dyslipidemia, hypertension, and obesity—marine-derived cryptides may have great potential for use as nutraceuticals and pharmaceuticals. Figure 5.1 presented the effects and mechanisms of action of marine peptides and hydrolysates in the pathogenesis of MetS. As shown, marine cryptides or hydrolysates can regulate dyslipidemia involved *in fine* in CVD. Hydrolysates from fish protein can also regulate blood glucose levels and hyperglycemia leading to type II diabetes. Moreover, they can reduce arterial hypertension and food intake, since they exhibit appetite suppressant activities. In addition, they may exhibit antioxidant activities, antiaggregation, and calcium-binding properties. Some can be considered multifunctional, since the same sequence can exhibit several biological activities. For example, very short cryptides can inhibit ACE and exhibit both antihypertensive and antioxidant properties. In a hydrolysate, it is not unrealistic to imagine that all peptides generated could easily target all the pathologies of MetS. Marine cryptides may thus become substitutes for synthetic drugs that lack the latter's deleterious side effects.

The initial step in the production of functional food ingredients, nutraceuticals, and pharmaceuticals containing FPH is the choice of raw material. The marine substrates for cryptide generation can be proteins that are already used for human consumption (filets, muscles) or proteins from the byproducts of marine processing (carcasses, heads, and skins, for example). Whatever the origin of the substrate, the sanitary quality and the availability of the resource are prerequisite conditions for the production of bioactive hydrolysate. The second important factor appears to be the choice of the protease leading to the desired peptide population. Numerous enzymatic preparations are commercially available, with various optimum pHs, temperatures, and treatment times for inactivation (Guerard *et al.* 2010). Since marine cryptides are low-molecular-weight peptides, membrane fractionation can be used to generate more potent bioactive fractions (Bourseau *et al.* 2009), but such fractionation should be associated in a fully integrated chain in order to avoid the discard of proteins or peptides. The process should also be reproducible and rapid, to allow recovery of cryptides. After checking the biological activity of whole hydrolysate, identification of the biologically active molecules and their molecular mode of action is necessary. The documentation of health claims is of importance, so biological activity detected by appropriate markers using *in vitro* tests must be accompanied by *in vivo* animal and eventually clinical human studies (Thorkelson *et al.* 2008). Marine cryptides with potent activity against MetS face a lack of human trials. Therefore, more studies into the bioavailability and the *in vivo* effects of marine cryptides, either alone or in the hydrolysate, will help the development of functional foods from the sea.

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